

Amphipathic polymer-mediated uptake of trehalose for dimethyl sulfoxide-free Human cell cryopreservation

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Abstract

For stem cell therapy to become a routine reality, one of the major challenges to overcome is their storage and transportation. Currently this is achieved by cryopreserving cells utilising the cryoprotectant dimethyl sulfoxide (Me₂SO). Me₂SO is toxic to cells, leads to loss of cell functionality, and can produce severe side effects in patients. Potentially, cells could be frozen using the cryoprotectant trehalose if it could be delivered into the cells at a sufficient concentration. The novel amphipathic membrane permeabilising agent PP-50 has previously been shown to enhance trehalose uptake by erythrocytes, resulting in increased cryosurvival. This work was extended to the nucleated human cell line SAOS-2. Using the optimum PP-50 concentration and media osmolarity, cell viability post-thaw was $60 \pm 2\%$. In addition, the number of metabolically active cells 24 h post-thaw, normalised to that before freezing, was found to be between $103 \pm 4\%$ and $91 \pm 5\%$. This was found to be comparable to cells frozen using Me₂SO. Although reduced (by $22 \pm 2\%$, $p = 0.09$), the doubling time was found not to be statistically different to the non-frozen control. This was in contrast to cells frozen using Me₂SO, where the doubling time was significantly reduced (by $41 \pm 4\%$, $p = 0.004$). PP-50 mediated trehalose delivery into cells could represent an alternative cryopreservation protocol, suitable for research and therapeutic applications.